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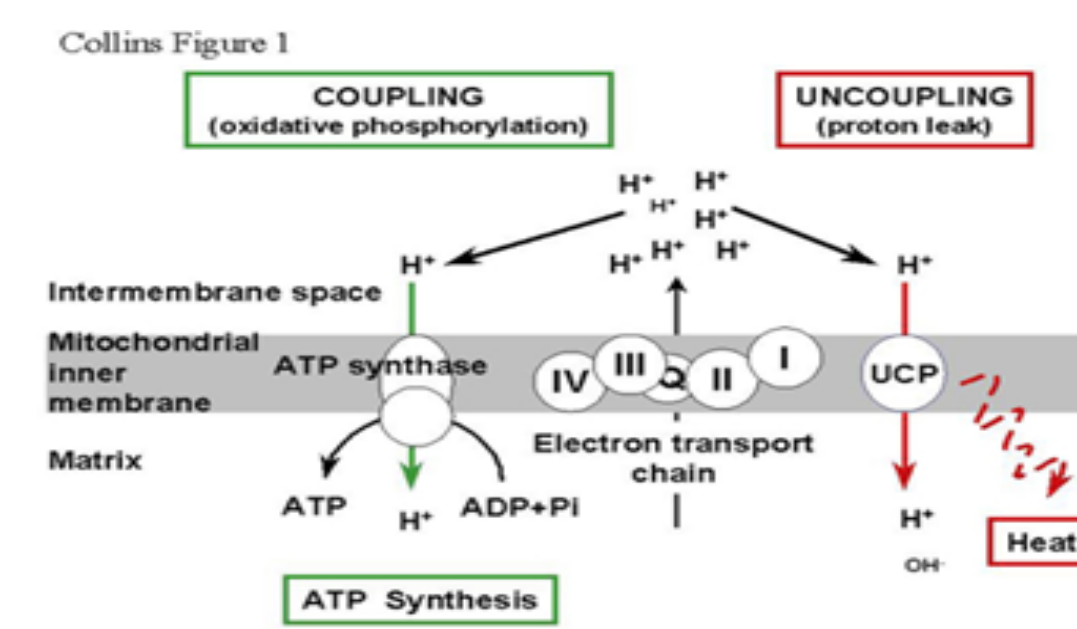
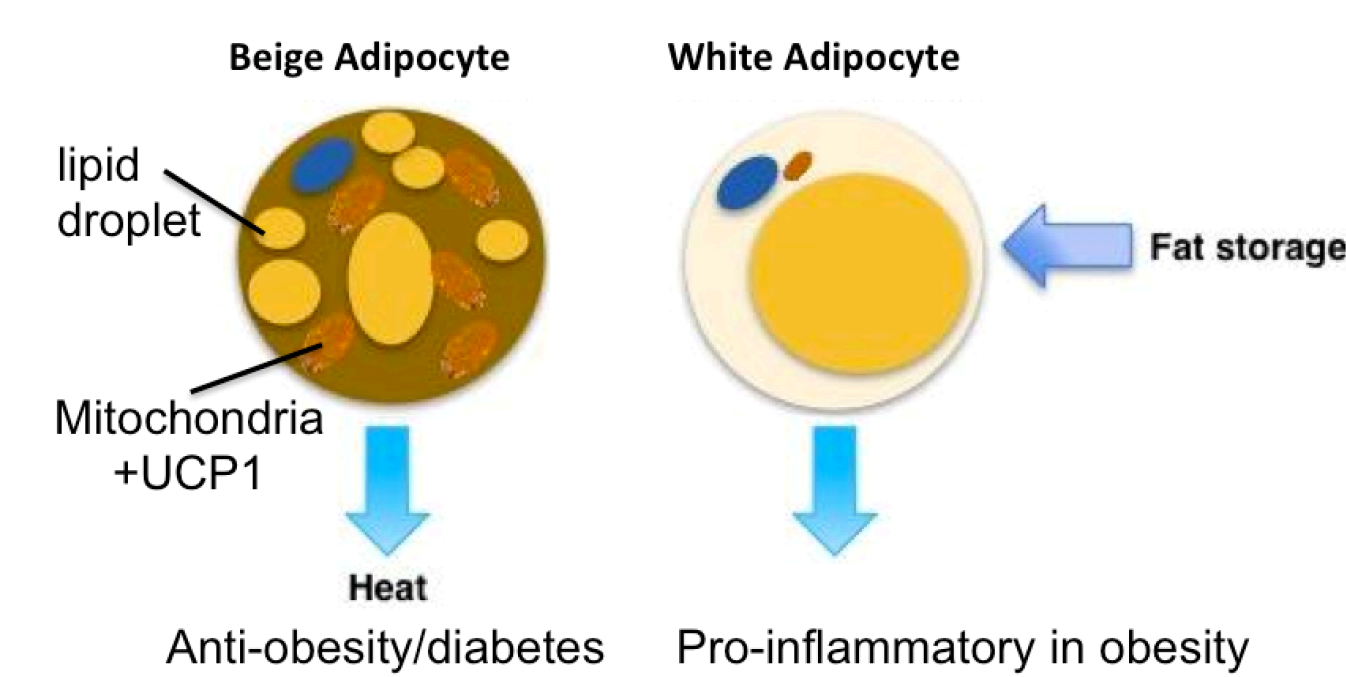
Analysis of anti-diabetic exosomes secreted from beige adipocytes

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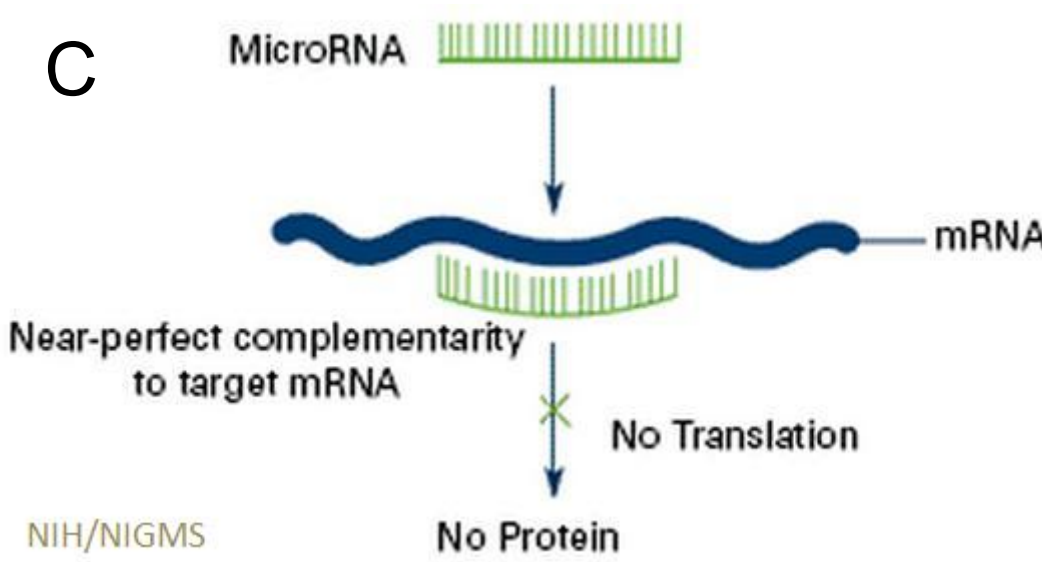
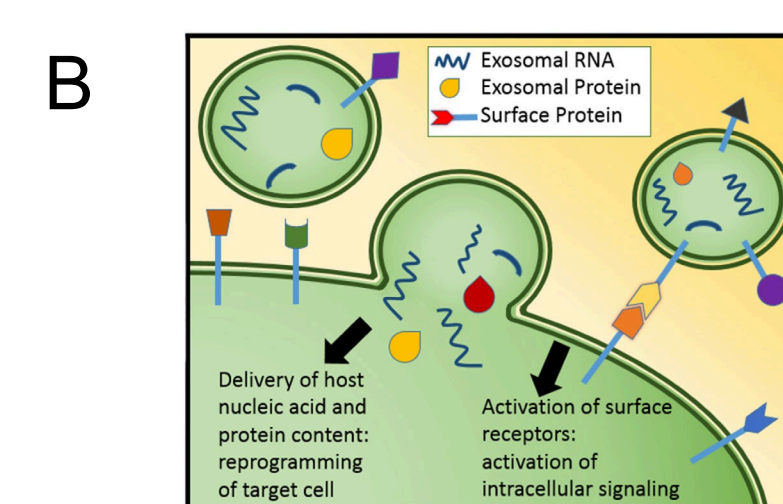
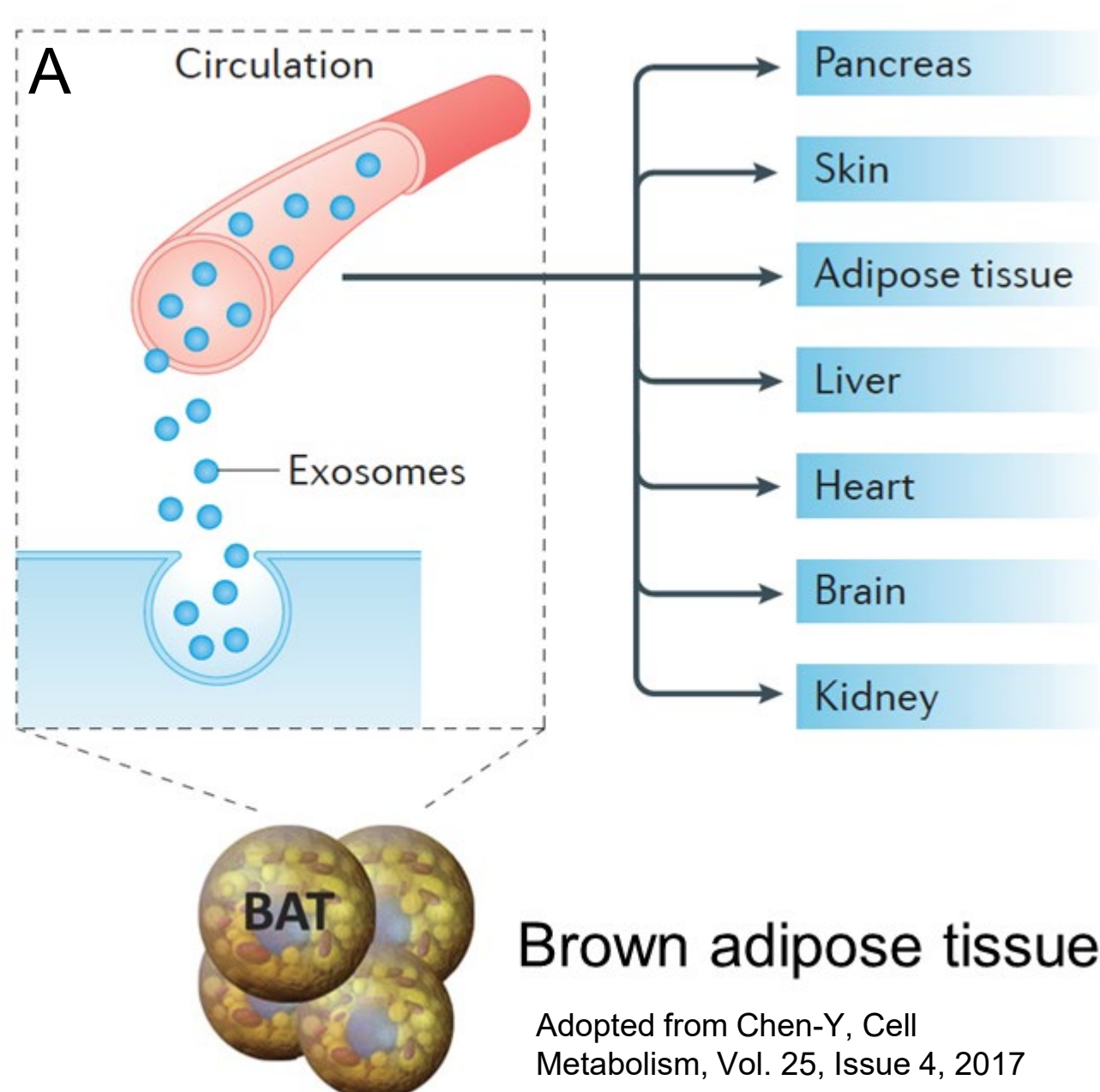
Abstract

Accumulation of excess fat in white adipose tissue is associated with an increase in risk for type 2 diabetes. Within white fat tissue resides a population of "beige" adipocytes that are activated by cold exposure and expend energy contained in fats, which is released as heat. Increasing energy expenditure through beige adipocyte activation has been shown to reduce diabetic symptoms in rodent models of obesity. However, activation of beige adipocytes through exposure of humans to cold temperatures is uncomfortable and likely not a realistic strategy to control body weight. In addition to its fat burning potential, secreted factors derived from activated beige adipocytes may enter the circulation and reduce diabetic symptoms such as insulin resistance in other tissues. The mechanisms by which these secreted factors act on distant tissues may in part be due to their transport inside extracellular vesicles, known as exosomes. Exosomes carry a diverse array of signaling molecules, including microRNAs that are transported and released into recipient cells and tissues. The goal of this project was to determine if beige adipocytes grown in cell culture secrete exosomes that contain microRNAs that may harbor anti-diabetic properties. Unexpectedly, we found that during the activation of beige adipocytes, secreted exosomes contain elevated expression of a number of microRNAs known to be negative regulators of beige adipocyte activation, including mir-27. This suggests that exosome secretion may be a way to increase beige adipocyte activation by decreasing the expression of specific microRNAs. Future testing of these microRNA candidates may translate to improved therapies for obese patients that develop diabetes.

Background

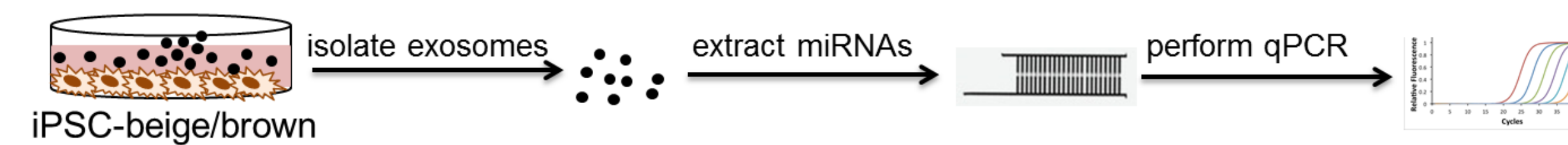


During weight gain, periods of prolonged overeating result in fat storage in white adipocytes, which can lead to inflammation, cellular stress, insulin resistance and eventually T2D. Unlike the energy storage function of white adipose tissue, beige adipocytes become metabolically activated in response to cold and burn calories by releasing energy stored in fats to generate heat, thereby providing mammals with protection from hypothermia. Therapeutic strategies are being sought to stimulate beige adipocytes to burn excess calories and combat obesity.



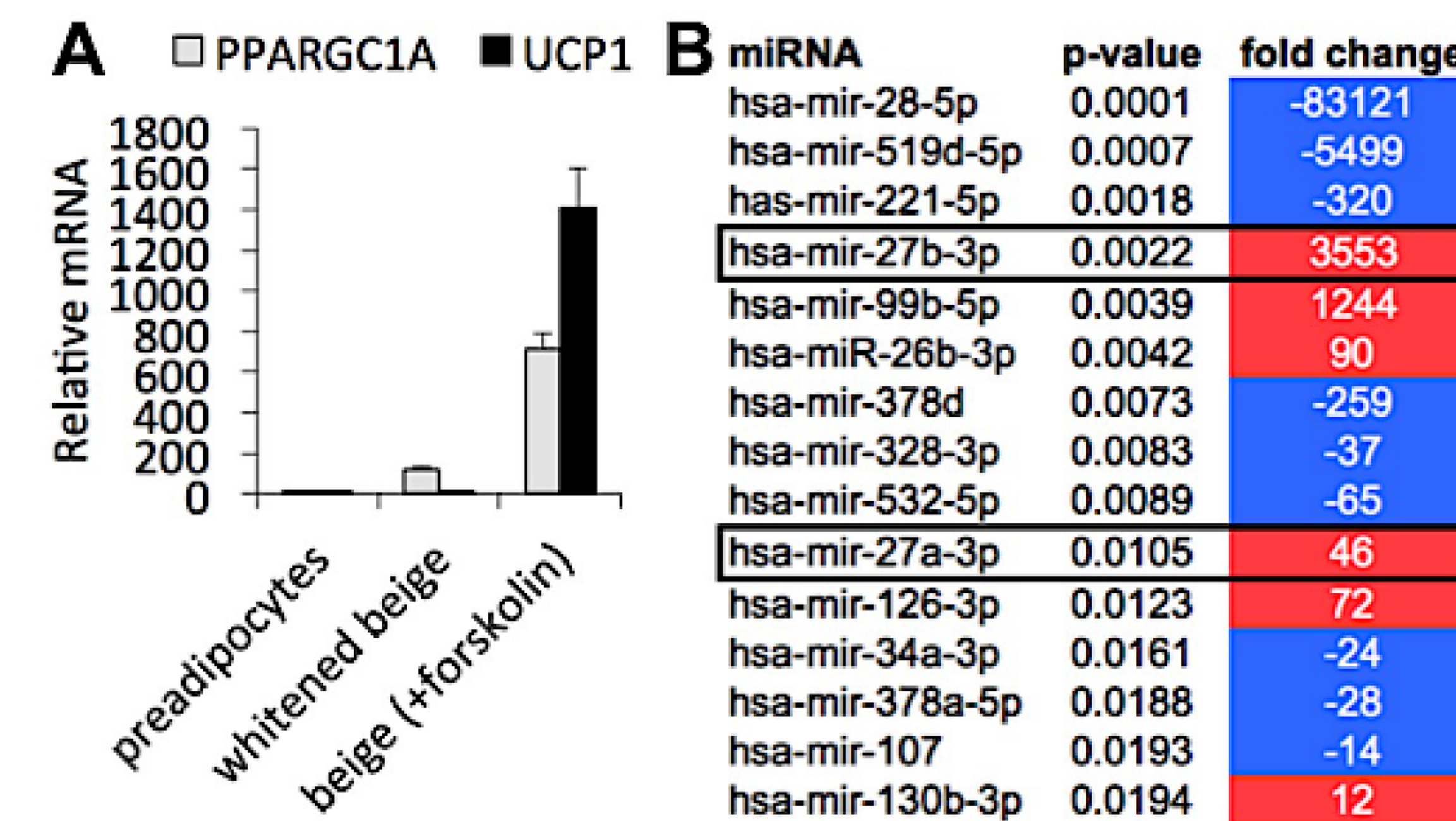
(A) Factors contained within exosomes secreted by brown and beige adipocytes may target and promote anti-diabetic effects in tissues affected by type 2 diabetes, such as the liver, white adipose tissue and others. (B) Exosomes carry a diverse array of signaling molecules, including microRNAs, proteins and lipids that are transported to recipient cells and tissues, potentially through direct homing via specific cell surface receptors. (C) microRNAs carried by exosomes can alter gene expression in distant tissues by binding to specific mRNA targets and silencing them.

Methods



- 1) Grow beige adipocytes in culture.
- 2) Isolate exosomes from conditioned medium.
- 3) Purify microRNA from isolated exosomes.
- 4) Make cDNA from exosomal microRNA.
- 5) Test expression of microRNAs previously shown to be present in exosomes secreted by beige adipocytes in vivo.

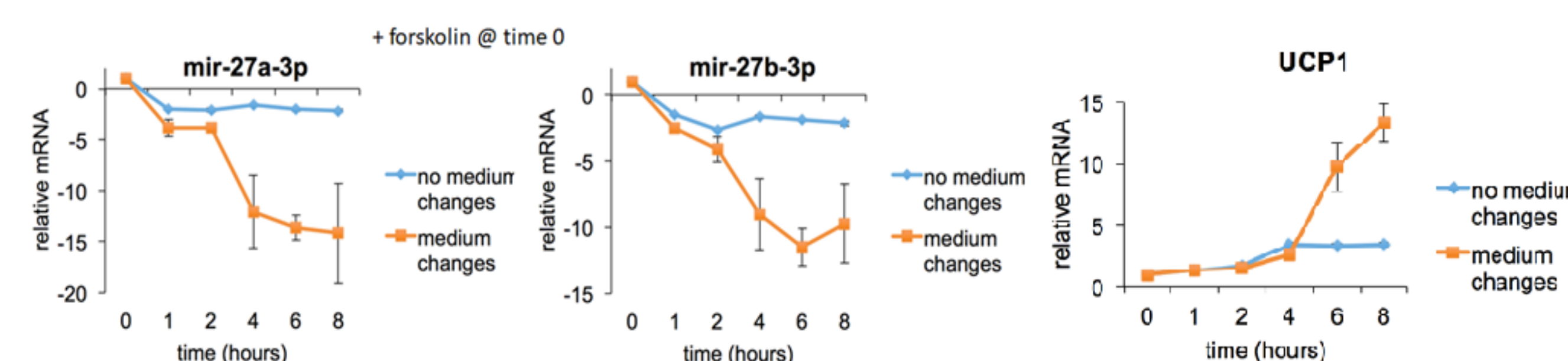
Results



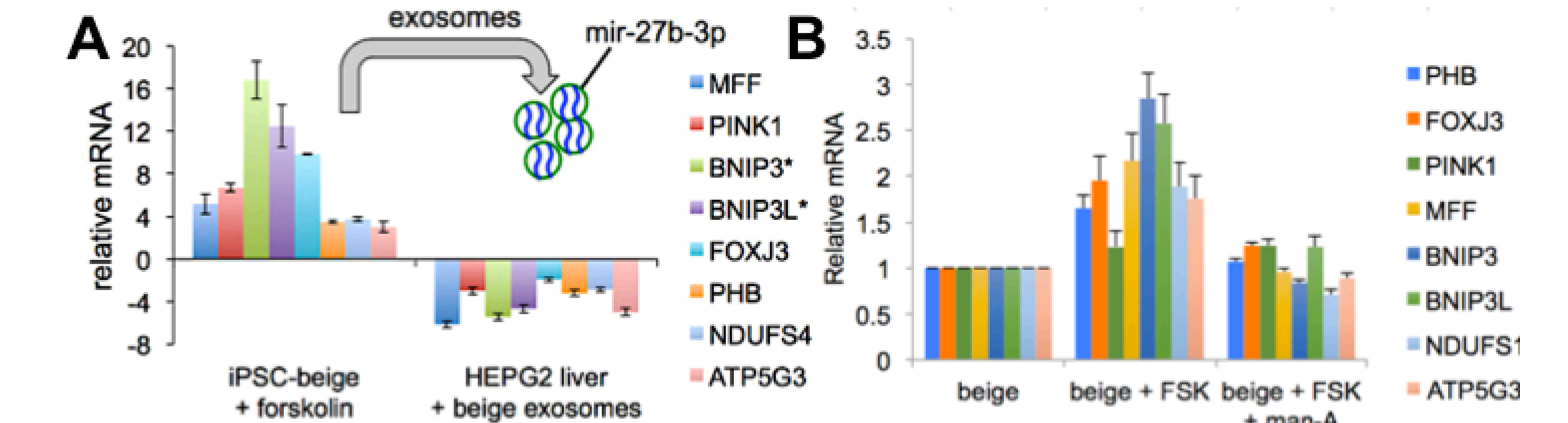
microRNA screen. (A) Activation of beige adipocytes in cell culture with forskolin. qPCR shown. (B) Differentially expressed miRNAs in exosomes from beige adipocytes after treatment with forskolin. 95 microRNAs were tested by qPCR. Cellular expression of mir-27a-3p and mir-27b-3p are known to be negative regulators of beige adipocyte activation and are increased in exosomes after activation.

An Alternative Hypothesis

Beige adipocyte activation is associated with rapid exosome-mediated clearance of mir-27, which promotes thermogenesis through increased mitochondrial biogenesis, membrane potential, and mitophagy-mediated clearance of damaged mitochondria.



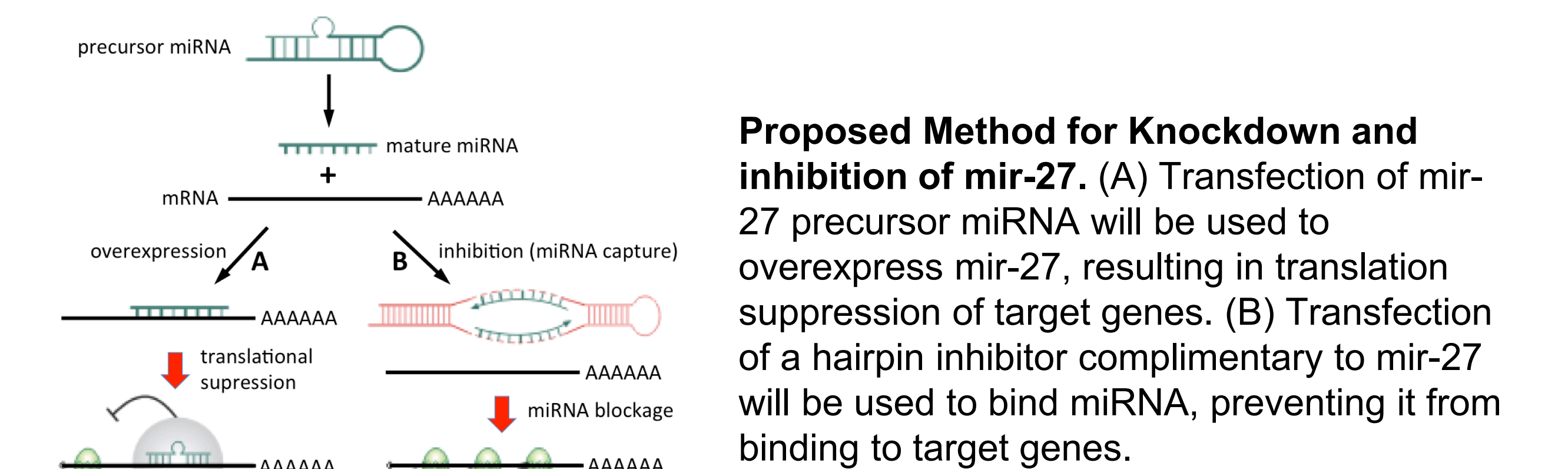
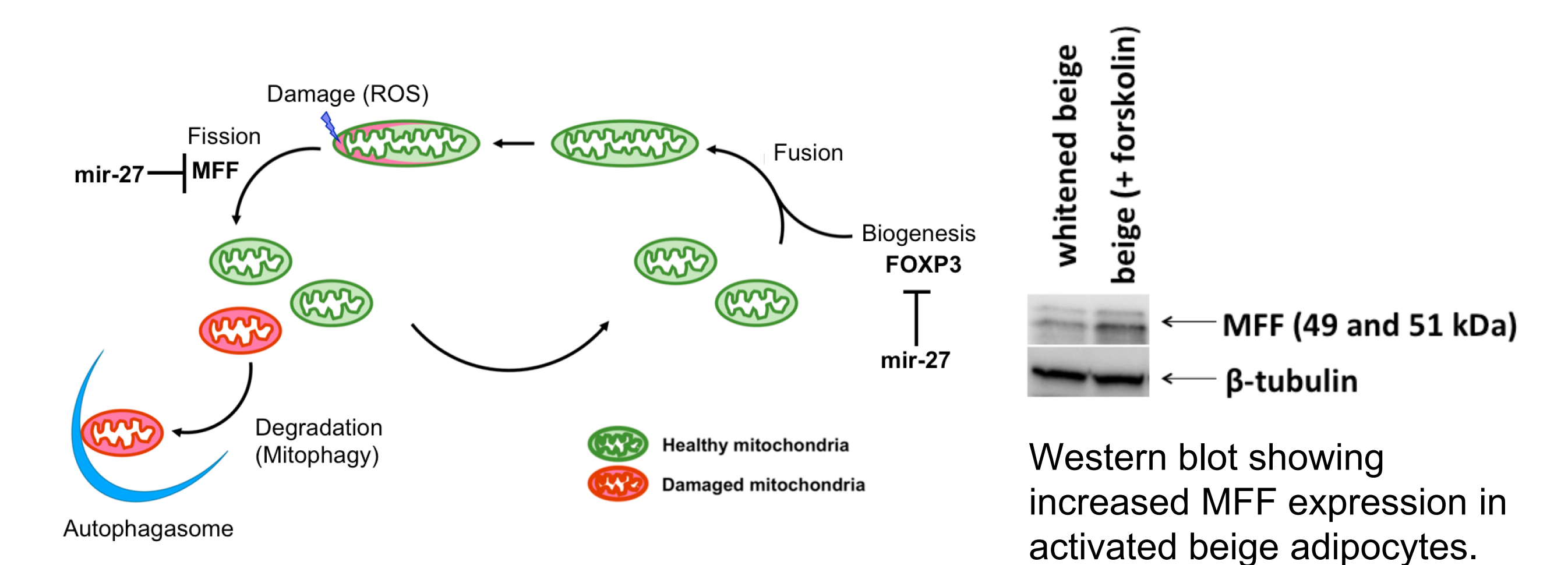
Continuous Clearing of Exosomes Accelerates Removal of mir-27 During Activation of Beige Adipocytes. Quantitative PCR analysis during beige adipocyte activation with and without culture medium changes. Cell culture medium was changed every hour during the forskolin treatment period, which caused mir-27a/b expression to decrease even further compared to no medium changes, where decreased cellular expression of mir-27a/b stabilizes after a few hours. Cellular expression of UCP1 also increased further, suggesting that exosome secretion may be associated with increased energy expenditure.



Beige Adipocyte Exosomes Differentially Regulate mir-27 Mitochondrial Target Genes. (A) Liver cells treated with exosomes from activated beige adipocytes show decreased expression of known and predicted mir-27a/b target genes. (B) mir-27a/b target gene expression is decreased in beige adipocytes activated in the presence of the exosome secretion inhibitor manumycin A (man-A).

Future directions

Hypothesized Role of mir-27 and Mitochondrial Fission Factor (MFF) in Mitochondrial Biogenesis and Degradation



Summary

Exosome secretion may provide a mechanism for down-regulation of negative regulators of thermogenesis. mir-27 may negatively repress activation of brown and beige adipocytes by targeting genes involved in promoting mitochondrial biogenesis and turnover. MFF may maintain mitochondria in a healthy state by removing those that are damaged. Exploitation of brown and beige adipocyte release of exosomes, their associated miRNAs or their target genes may be used to develop new therapies for the treatment of obesity and metabolic syndrome.

Acknowledgements

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